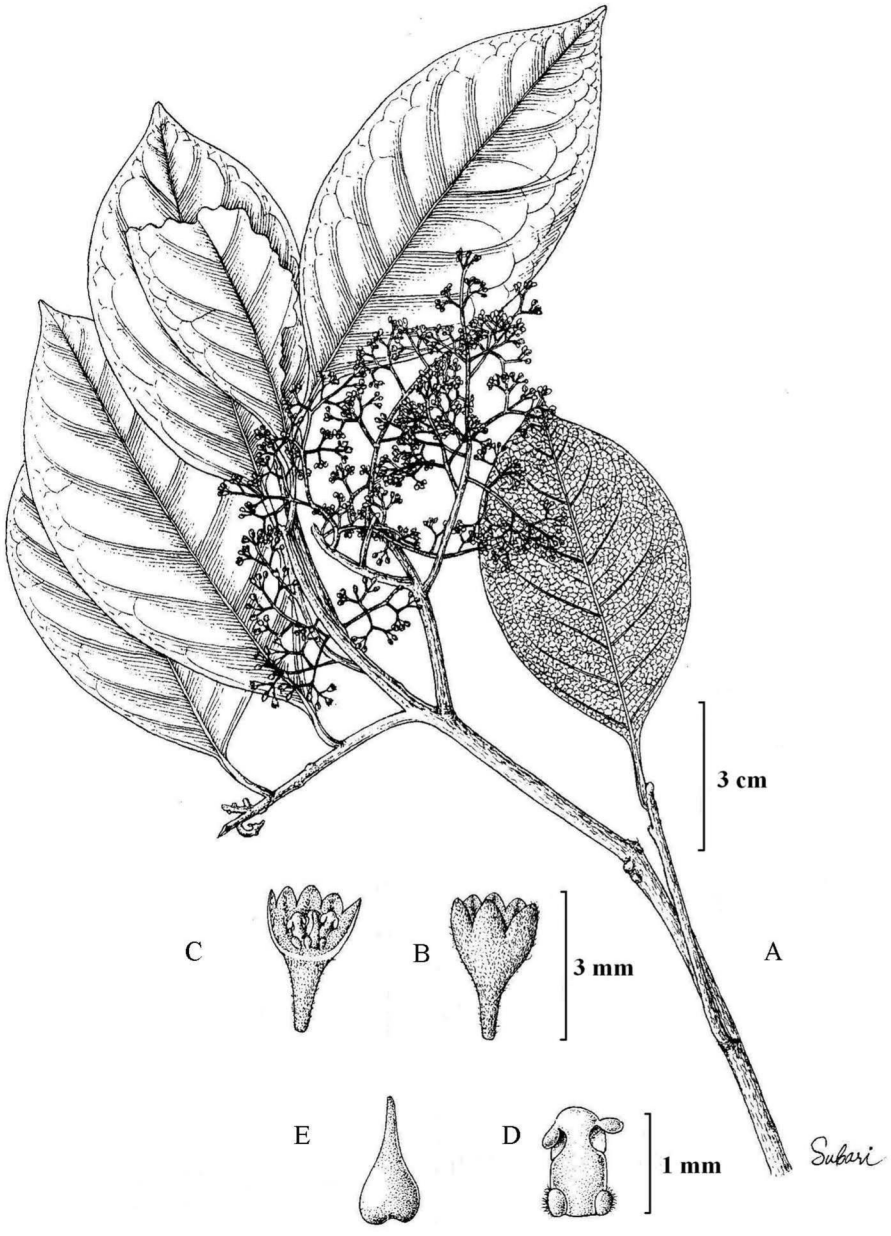
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A JOURNAL ON TAXONOMIC BOTANY, PLANT SOCIOLOGY AND ECOLOGY

ISSN 0034 – 365 X



REINWARDTIA

13 (4)

REINWARDTIA

A JOURNAL ON TAXONOMIC BOTANY, PLANT SOCIOLOGY AND ECOLOGY

Vol. 13(4): 317 — 391, December 20, 2012

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REINWARDTIA
Vol 13, No 4, pp: 357–362

DICKSONIA TIMORENSE (DIKSONIACEAE), A HEMI-EPIPHYTIC NEW SPECIES OF TREE FERN ENDEMIC ON TIMOR ISLAND, INDONESIA

Received March 22, 2012; accepted July 28, 2012.

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ABSTRACT
ADJIE, B., KURNIAWAN, A., SAHASHI, N. & WATANO, Y. 2012. *Dicksonia timorense* (*Diksoniaceae*), a hemi-epiphytic new species of tree fern endemic on Timor Island, Indonesia. Reinwardtia 13(4): 357–362. — *Dicksonia timorense* B. Adjie is described and illustrated as an endemic new species from Timor Island, Indonesia. Population, hemi-epiphytic trait and phylogenetic relationship based on cpDNA sequences are discussed.

Keywords: *Dicksonia*, Timor, Indonesia, new species, endemic, hemi-epiphytic, cpDNA.

ABSTRAK
ADJIE, B., KURNIAWAN, A., SAHASHI, N. & WATANO, Y. 2012. *Dicksonia timorense* (*Diksoniaceae*), jenis baru paku pohon hemi-epifitik yang endemik dari Pulau Timor, Indonesia. Reinwardtia 13(4): 357–362. — *Dicksonia timorense* B. Adjie dipertelakan dan diilustrasikan sebagai jenis baru endemik dari Pulau Timor, Indonesia. Studi tentang populasi, aksesi hemi-epifitik serta hubungan kekerabatan filogeni berdasarkan cpDNA disajikan.

Kata kunci: *Dicksonia*, Timor, Indonesia, jenis baru, endemik, hemi-epifitik, cpDNA.

INTRODUCTION

The family *Dicksoniaceae* sensu Kubitzki (1990) currently recognized is not monophyletic (Koral *et al*., 2006). Smith *et al*. (2006) delimit *Dicksoniaceae* to include only three genera (*Calochlaena*, *Dicksonia*, and *Lophosoria*) as monophyletic group with *ca*. 30 spp. Among the genera of the family, *Dicksonia* has a tree-like form. *Dicksonia* L'Hér. Comprises 20–25 species, distributed from Central and South America, throughout Pacific to the Samoa, New Caledonia, New Guinea, Australia to New Zealand; most species occur in Malesia in montane forest (Kubitzki, 1990; Large & Braggins, 2004). In Indonesia, four species known so far *D. blumei* (Kunze) Moore, *D. moliis* Holttum, *D. archboldii* Copel. and *D. lanigera* Holttum (Holttum, 1963). *Dicksonia blumei* distributed through Sumatra, Java and Central Celebes; *D. mollis* distributed in NE. Borneo, Central Celebes and Philippines; *D. archboldii* only known from the type collection of West New Guinea and Mt. Arfak; and *D. lanigera* distributed in West and East New Guinea.

Two expeditions by Arinasa and Lugrayasa in 1995 and 1996 to Mutis Nature Reserve, Timor islands discovered a small population of *Dicksonia* with about 50 plants. They brought 18 young plants to Bali Botanic Garden, and 14 of them are well established at Cyathea Park. Morphological examinations have provided important clues that the tree fern is distinct species to described known species. Moreover, DNA sequences can provide robust evidences for species identity as well as their phylogenetic relationships among species.

Taxon Sampling, DNA isolation, amplification, and sequencing

Genomic DNA was extracted from silica-dried, leaf material using a modified Doyle & Doyle (1987). Two regions of cpDNA (*rbc*L gene and *trn*L (UAA) 5′exon to *trn*F (GAA), later called *trn*L -F IGS) were amplified separately using the polymerase chain reaction (PCR), following established protocols (Hasebe *et al.*, 1994; Taberlet *et al.*, 1991). The PCR products were purified using the Geneclean II kit (Qbiogene, Irvine, CA, USA) after

electrophoresis in 1% agarose gel and used as templates for direct sequencing. Sequencing reactions were carried out with a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were processed using ABI 310 Genetic Analyzer (Applied Biosystems). Sequence fragments were analyzed using the Sequencing Analysis v5.2 (Applied Biosystems) and assembled by use of SeqScape v2.5 (Applied Biosystems). DNA sequences were deposited in GenBank as part of this study.

For this purposes several *rbc*L sequences of *Dicksoniaceae* family was downloaded from GenBank and used as ingroup. *Cibotium barometz* was chosen as outgroup for rooting based on previous study (Korall *et al.*, 2006) as in Table 1.

Alignment and phylogenetic analysis

The *rbc*L sequences were aligned automatically by use of ClustalW (Higgins *et al.*, 1994). Aligned sequence was then used for phylogenetic analysis using MEGA 5 software (Tamura *et al.*, 2011). The distance (Neighbor-Joining tree) and parsimony (Maximum Parsimony) methods were used to construct the phylogenetic tree. Confidence values were calculated with bootstrap procedure (Felsenstein, 1985) in 1000 replicates.

A 1229 bp of *rbc*L gene was successfully sequenced, while a 361 bp sequenced from *trn*L-F IGS. Alignment of *rbc*L sequences produce 1183 bp with no indels that are used for analysis. The topology of NJ tree was identical with MP tree as presented with bootstrap value shown along the

Table 1. Taxon sample and GenBank accession number for phylogenetic analysis.

No.	Species	*rbc*L	*trn*L-F
1.	*Dicksonia timorense*	HQ334990*	AY626843*
2.	*Dicksonia antarctica*	DAU05919	-
3.	*Dicksonia gigantea*	GI99640797	-
4.	*Dicksonia arborescens*	AM177340	-
5.	*Dicksonia squarrosa*	AM177344	-
6.	*Dicksonia lanata*	AM177343	-
7.	*Dicksonia thyrsopteroides*	AM177345	-
8.	*Dicksonia fibrosa*	AM177341	-
9.	*Dicksonia blumei*	HQ334991*	AY626841*
10.	*Lophosoria quadripinnata*	AF101303	-
11.	*Calochlaena dubia*	U05615	-
12.	*Calochlaena villosa*	AM177327	-
13.	*Cibotium barometz*	AM177328	-

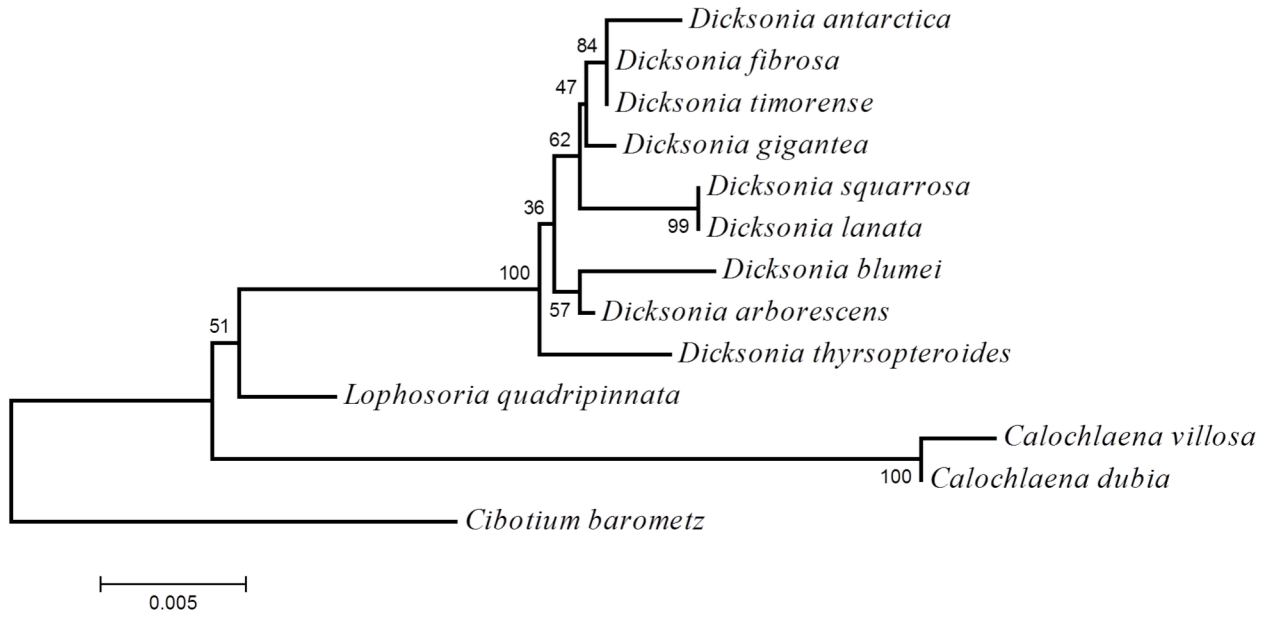


Fig. 1. The phylogenetic tree showing the position of *Dicksonia timorense*. The tree was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the Bootstrap test (1000 replicates) is shown.

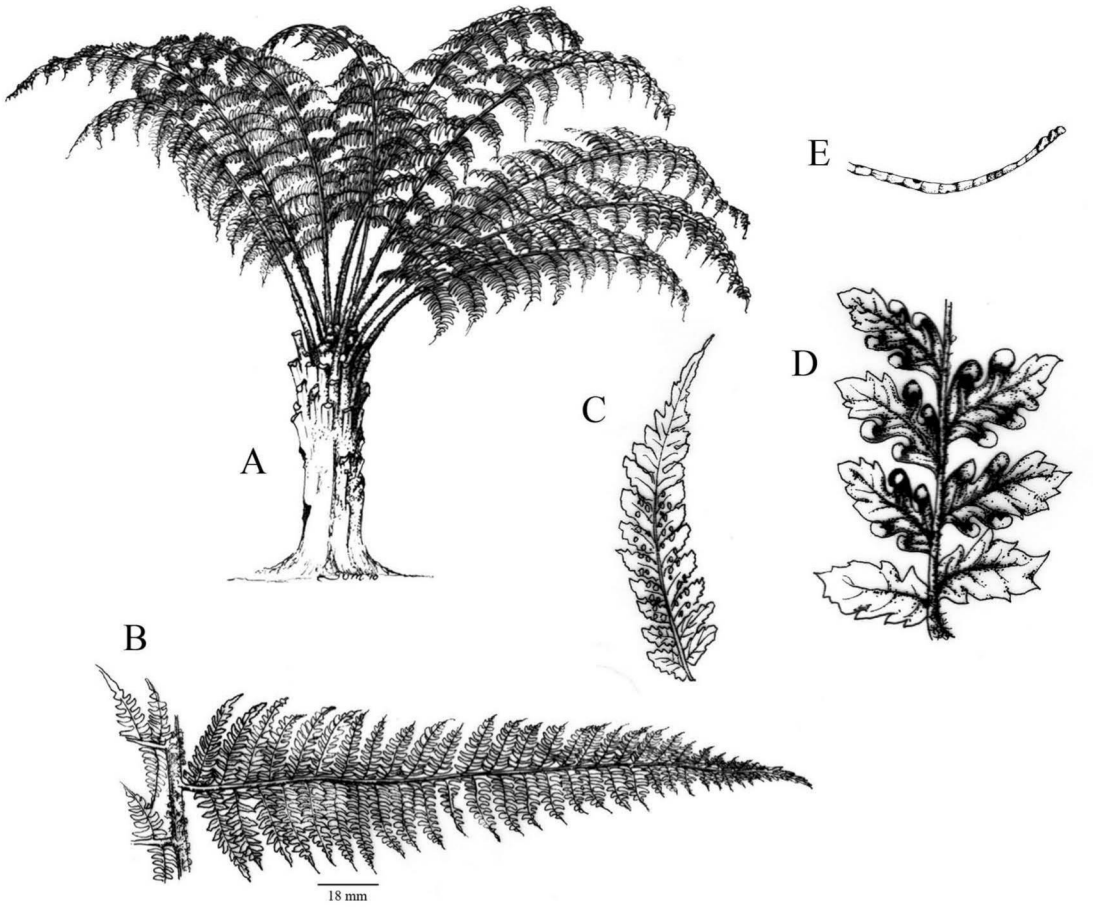


Fig. 2. *Dicksonia timorense* B. Adjie illustrated. A. Young plant in cultivation; B. Pinnae; C. Pinnules; D. Pinnules with sori; E. Macroscopic hair.

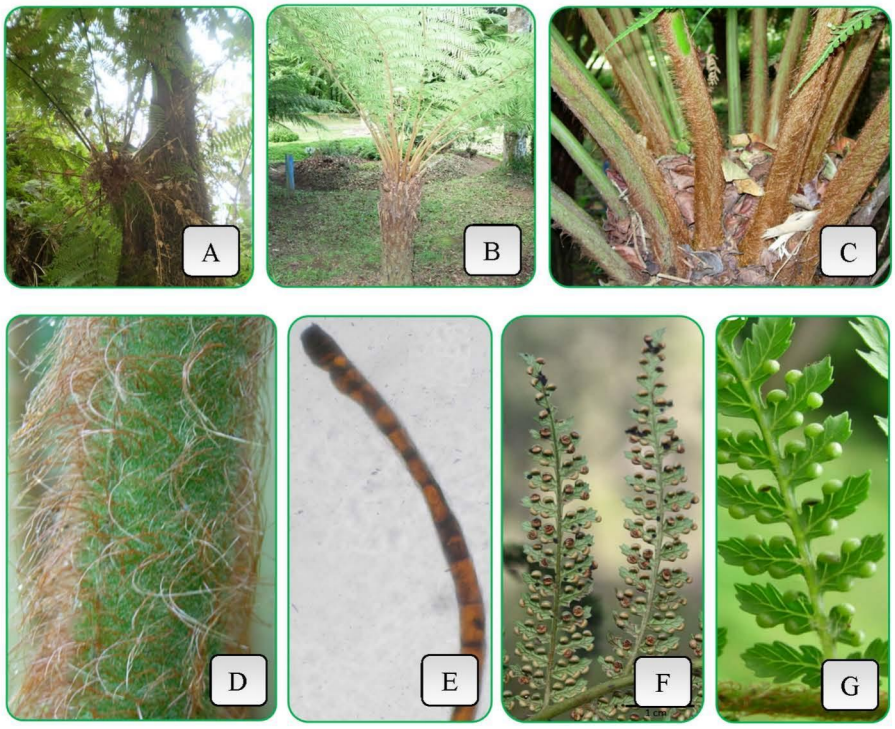


Fig. 3. *Dicksonia timorense* B. Adjie. A. Young plant in natural habitat; B. Type specimens in cultivation; C. Top part of trunk; D. Hairs on stipe; E. Macroscopic hair; F. Pinnules with mature sori; G. Pinnule with young sori.

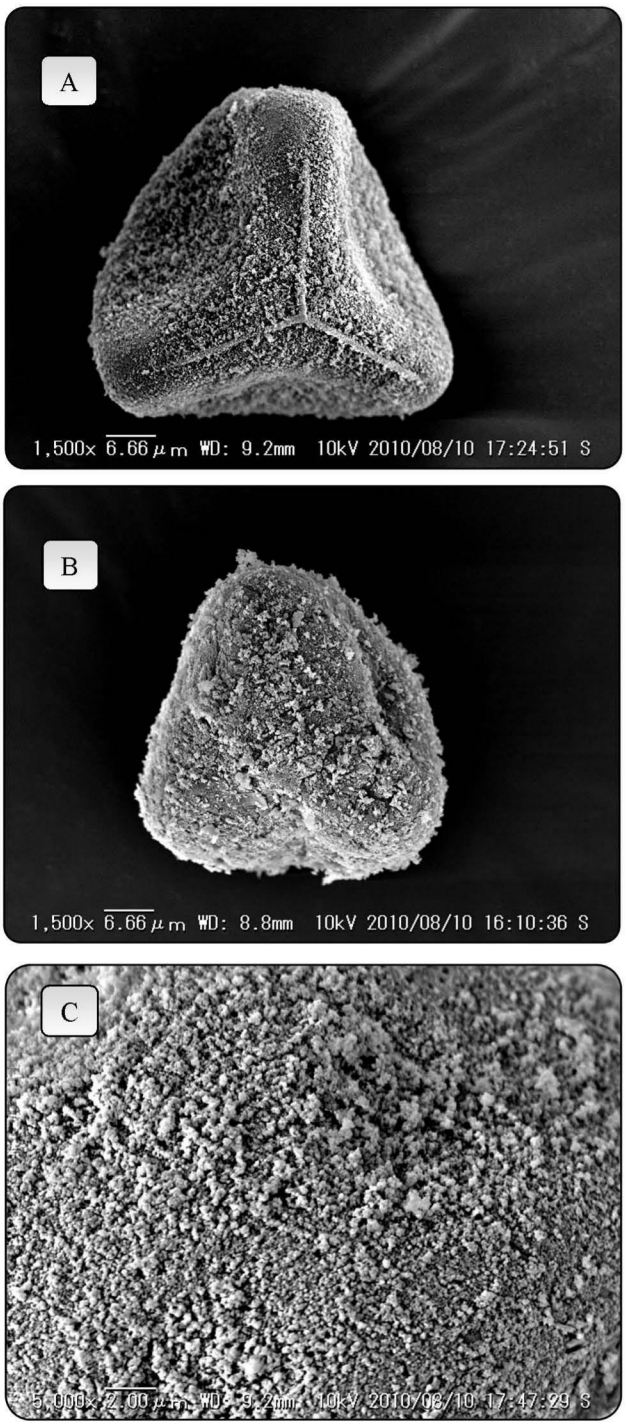


Fig. 4. Spores of *Dicksonia timorense*. A. Polar view, proximal face depressed between partly obscured laesurae; B. Polar view, distal face densely granulates, somewhat shrink margin; C. Part of distal face, showing detail, granulate surface.

branches, and the core of *Dicksoniaceae* is monophyletic and clustered into three monophyletic clades. A close relationship among these taxa was previously shown by Korall *et al.* (2006). *Dicksonia timorense* grouped together with *D. antartica* from Australia and *D. fibrosa* from New Zealand. The *rbc*L sequences of *D. timorense* and *D. fibrosa* are similar making it difficult to identify based on the sequence (Fig. 1). Unfortunately, the *trn*L-F sequence of *D. fibrosa* is unavailable from the GenBank so that we are unable to compare the sequence with *D. timorense*.

TAXONOMY SECTION

Dicksonia timorense B. Adjie *sp. nov.* — Fig. 2 & 3

Tree fern with pale brown hairs covered stipe base, closely resembles to *D. fibrosa* in frond morphology and persistency, differ in stipe length and number of sorus per pinnule lobe. It is readily distinguished from *D. blumei* by the hair color and spore morphology. — Type: Indonesia, Timor Island, Nusa Tenggara Timur, Mutis Nature Reserve, Bukit Lelofui, 1760 m, cultivated in Bali Botanic Garden, Bayu Adjie *BA653* (Holotype the Herbarium of Bali Botanic Garden; Isotype BO, K).

Tree fern with trunk up to 5 m in height, diameter 20 cm, number of fronds up to 36, stipe bases persistent; stipe covered by pale brown hairs 15–20 mm long; lower part of stipe and main rachis green. *Frond* total length 170–230 cm; stipe 33–55 cm long, width 1.5–2 cm, thickness 1.5 cm; rachis 130–170 cm long; distance from basal pinna to next pinna 11–13 cm, number of pinna 44–46 including final terminal pinna. *Pinna* longest pinna 28–36 cm long, 8–11 from base position on rachis, number of sori per pinnule lobe 3–5 rarely 6, large, globose, marginal, and under the hooded edges of the lobes; indusia oblong to circular; lobes not reduced when fertile; fertile pinnae 7–10; basal pinnae not reduced but much smaller, 10–22 cm long, sterile; *pinnules* to 50 by 10 mm; costules of tertiary leaflets 5 mm apart; largest fertile tertiary leaflets lobed throughout almost to the costule, with 2 pairs rarely 3 of soriferous lobes, the lowest lobes usually bilobulate with forked vein, rest veins in pinnule lobes pinnate or simple. *Spores* yellow, trilete, proximal face depressed between partly obsured laesurae with distal face densely granulate, margins somewhat shrunk (as can be seen in Fig. 4).

Distribution. Indonesia, Nusa Tenggara Timur, Timor Island: endemic to Mt. Mutis Nature Reserve (NR) at Bukit Lelofui.

Habitat. Upper montane forest, shaded area; *ca.* 1760 m asl. in a valley between two ridges separated by creek.

Population. *Dicksonia timorense* is currently only known from a single population from Mutis NR. Recently, the first and second authors visited the

species natural habitat after it was first discovered 15 years ago. The habitat is almost undisturbed as described in Arinasa (2007), the individual number has increased from 50 to *ca*. 68 adult plants and eight juveniles. However, this fragile ecosystem could be easily disturbed due to extensive cattle and horse grazing.

Etymology. The name refers to the island of Timor where the species is found.

Notes. *Dicksonia timorense* B. Adjie is recognized by its pale brown hairs that are easily removed and abundant fronds. In cultivation the sori are very rare, in one tree only 1–3 fronds have sorus; sometimes there is no fertile frond at all. However, in the natural populations sori are abundantly found on fronds. *Dicksonia timorense* closely resembles New Zealand's *D. fibrosa*, notably in frond morphology, persistent and similarity in *rbc*L sequence. It differs from *D. fibrosa* in the number of sori per pinnule lobe (mostly six) and the life habit (solitary). *Dicksonia fibrosa* from the Chatham islands bear strong similarity to the Australian *D. antartica* (Large & Braggins, 2004); however, there are three different nucleotides substitution in the *rbc*L sequences. *Dicksonia timorense* is readily distinguished from *D. blumei* by the hair color (red-brown shining hairs) and spore morphology (distal face showing irregular lumina and small pits on muri, unpublished data).

In recent observations of their natural habitat, the species has a remarkable hemi-epiphytic phenomenon: young plants are always deeply attached to and growing on *Cyathea* trunks about 1 m from ground level; no young plants found growing on soil. Once the *Dicksonia* roots reach the ground, the *Cyathea* fronds die and fall. And then, after the roots and trunk of *Dicksonia* are strongly attached in the ground, the *Cyathea* trunk will fall. In some adult trees it was observed that the base of the *Cyathea* host trunk remain attached to the *Dicksonia* trunk. We assume this hemi-epiphytic habit to gain some "ecological" advantage from the host. The presence



Fig. 5. Hemi-ephiphitic habitat of *Dicksonia timorense*. A. Young sporophyte attached to *Cyathea* trunk; B. *Dicksonia*'s roots reach the ground; C. *Cyathea* become dead (arrow); D. Adult sporophyte.

of hemi-epiphytic on tree fern trunk has been reviewed by Page & Brownsey (1986), but mostly by flowering plants such as *Ackama rosaefolia* (*Cunoniaceae*), *Metrosideros robusta* (*Myrtaceae*), *Pseudopanax arboreus*, *P. edgerleyi* (*Araliaceae*), and *Weinmannia racemosa* and *W. silvicola* (*Cunoniaceae*). Therefore, the hemi-epiphitic habit in *Dicksonia* is firstly described. The hemi-epiphitic habit can be seen in Fig. 5.

ACKNOWLEDGEMENT

We wish to thank Dr. Robert Johns for drawing our attention to this plant during a recent visit to Bali. We especially thank Director of Bali Botanic Garden who provided facilities, IBK Arinasa and IDP Darma the two chiefs on the Mutis expedition in 1995 and 1996. We thank Dr Tadashi Kajita (Chiba University) for handling spores. We are grateful to the head of BBKSDA Kupang for research permit, park rangers Peter Didok and Soleman Toto, and Siwa "Siu" Anin our best local guide; I Made Sudi, I Ketut Sandi for field assistance. Special thanks to I Made Sumerta for line drawing. The field trip was funded by research incentive grant for researcher and engineer no. 77/M/Kp/II/2011 to BA.

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